2-1-2012

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Recommended Citation

Nagarajan, V., & Smith, A. (2012). Ethylene's role in phosphate starvation signaling: More than just a root growth regulator. *Plant and Cell Physiology, 53* (2), 277-286. https://doi.org/10.1093/pcp/pcr186

PCP
PLANT & CELL PHYSIOLOGY

Ethylene's Role in Phosphate Starvation Signaling: More than Just a Root Growth Regulator

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(Received October 24, 2011; Accepted December 15, 2011)

Phosphate (Pi) is a common limiter of plant growth due to its low availability in most soils. Plants have evolved elaborate mechanisms for sensing Pi deficiency and for initiating adaptive responses to low Pi conditions. Pi signaling pathways are modulated by both local and long-distance, or systemic, sensing mechanisms. Local sensing of low Pi initiates major root developmental changes aimed at enhancing Pi acquisition, whereas systemic sensing governs pathways that modulate expression of numerous genes encoding factors involved in Pi transport and distribution. The gaseous phytohormone ethylene has been shown to play an integral role in regulating local, root developmental responses to Pi deficiency. Comparatively, a role for ethylene in systemic Pi signaling has been more circumstantial. However, recent studies have revealed that ethylene acts to modulate a number of systemically controlled Pi starvation responses. Herein we highlight the findings from these studies and offer a model for how ethylene biosynthesis and responsiveness are integrated into both local and systemic Pi signaling pathways.

Keywords: Ethylene • Local • Phosphorus deficiency • Sensing • Systemic.

Abbreviations: ACC, 1-aminocyclopropane-1-carboxylate; ACO, ACC OXIDASE; ACP5, ACID PHOSPHATASE TYPE 5; ACS, ACC SYNTHASE; AP2/ERF, APETALA2/ETHYLENE RESPONSE FACTOR; At4, *Arabidopsis thaliana* cDNA 4; AVG, aminoethoxyvinylglycine; CHL1, CHLORATE RESISTANT 1; CTR1, CONSTITUTIVE TRIPLE RESPONSE 1; EBF, EIN3-BINDING F-BOX; EIL1, EIN3-LIKE 1; EIN2, ETHYLENE INSENSITIVE 2; EIN3, ETHYLENE INSENSITIVE 3; ETR1, ETHYLENE RESPONSE 1; FRY1, FIERY1; hps2, hypersensitive to phosphate starvation 2; IPS1, Induced by Phosphate Starvation 1; MYB, myeloblastosis; PAP, purple acid phosphatase; PHL1, PHR1-LIKE 1; PHO1, PHOSPHATE 1; PHO2, PHOSPHATE 2; PHR1, PHOSPHATE STARVATION RESPONSE 1; Pht1, PHOSPHATE TRANSPORTER 1; Pi, inorganic phosphate; PSR, phosphate starvation response; qRT–PCR, quantitative reverse transcription–PCR; RAF, rapidly accelerated fibrosarcoma; RNS1, RIBONUCLEASE 1; *ron1-1*, *rotunda1-1*.

Introduction

Phosphate (Pi) availability in most soils is seldom adequate for optimal plant growth. Hence, plants have evolved many adaptations to tolerate Pi limitation that are initiated by a series of molecular, physiological and morphological changes. These adaptations to Pi deficiency are modulated via both local and systemic signaling pathways (H. Liu et al. 1998, Burleigh and Harrison 1999, Linkohr et al. 2002, Franco-Zorrilla et al. 2005, Sanchez-Calderon et al. 2006, Thibaud et al. 2010). Local sensing of low Pi in soil primarily initiates root morphological changes aimed at foraging available Pi. These root alterations include cessation of primary root growth and increased proliferation of root hairs and lateral roots (also adventitious and cluster/proteoid roots; Vance et al. 2003, Lynch 2011, Peret et al. 2011). The transduction of a low Pi, root-derived signal to shoots initiates systemic signaling pathways, which utilize multiple factors (e.g. transcription factors and microRNAs) to control the expression of a number of Pi starvation response (PSR) genes (Yang and Finnegan 2010, Chiou and Lin 2011). The bulk of these PSR genes facilitate Pi acquisition, mobilization and redistribution (Thibaud et al. 2010).

The root developmental changes initiated by local Pi sensing result from complex interactions among Pi availability, phytohormone fluxes and sugar signaling, as well as the availability of other nutrients (Franco-Zorrilla et al. 2005, Liu et al. 2005, Jain et al. 2007, Karthikeyan et al. 2007, Svistoonoff et al. 2007, Ward et al. 2008, Jain et al. 2009). Phytohormones, namely auxin, cytokinins, gibberellic acid, ABA, strigolactones and ethylene, have all been implicated in modulating low-Pi-induced changes to root system architecture (Chiou and Lin 2011). In particular, auxin and ethylene have well-documented roles in modulating root system architecture changes during Pi deficiency. Auxin appears to enhance lateral root initiation (Lopez-Bucio et al. 2002, Perez-Torres et al. 2008), whereas ethylene regulates root hair growth and root elongation (Borch et al. 1999, Ma et al. 2003, Zhang et al. 2003). In contrast to their roles in local, root morphological PSRs, involvement of phytohormones in modulating systemically controlled PSRs has

Plant Cell Physiol. 53(2): 277–286 (2012) doi:10.1093/pcp/pcr186, available online at www.pcp.oxfordjournals.org

been unclear. Recent efforts to understand the interaction between Pi homeostasis and ethylene have provided strong evidence supporting the involvement of ethylene in systemic Pi signaling pathways in addition to Pi-dependent root modifications. Herein we highlight these recent reports, as well as provide an update on ethylene's role in local Pi signaling. We propose a model in which ethylene plays a prominent role in modulating both local and systemic responses to Pi starvation.

Local Pi responses

Local Pi sensing

The plant root system with its highly plastic traits is instrumental in acquiring nutrients from the surrounding environment. Signals arising from the roots can provide the shoots with an early warning of low nutrient availability in the external environment. Several studies have indicated that nutrients such as Pi, with heterogeneous distribution in the rhizosphere, are sensed by a root-localized mechanism (Forde and Lorenzo 2001). It can be hypothesized that local sensing of external Pi is achieved by two means: intracellularly following import or extracellularly on the root cell plasma membrane (**Fig. 1** [1]). An internal sensing mechanism is supported by the observation that phosphate-sequestering metabolites, which caused transient decreases in cytosolic Pi levels, were able to initiate PSRs (Kock et al. 1998). Although a mechanism for extracellular sensing of Pi has not been identified, it is possible that high-affinity Pi transporters act as sensors of Pi at the root cell plasma membrane. A recent study demonstrated that the yeast Pho84p high-affinity Pi transporter functions as a transceptor, a transporter that senses nutrient levels and activates signaling events (Popova et al. 2010). This phenomenon was identified for the Arabidopsis nitrate transporter, CHL1/NRT1.1, which functions both as a transporter and as a sensor of nitrate, in part via changes in phosphorylation states (Ho et al. 2009). The two major Arabidopsis transporters involved in Pi uptake are the PHOSPHATE TRANSPORTER1 (Pht1) family members, Pht1;1 and Pht1;4 (Shin et al. 2004). Both of these transporters are localized to the epidermal cells of the root hair (Karthikeyan et al. 2002, Mudge et al. 2002), an appropriate site for Pi perception. It is plausible that plant Pht1 members, such as Pht1;1 and Pht1;4, could sense a range of extracellular Pi concentrations by the alteration of phosphorylation states or other post-translational modifications. However, experimental evidence is needed to support this hypothesis.

Ethylene's role in local Pi responses

Under low Pi conditions, local Pi sensing leads to altered expression of many PSR genes and the initiation of several adaptations aimed at remodeling root system architecture to more efficiently acquire Pi (**Fig. 1** [2, 3]; Thibaud et al. 2010, Chiou and Lin 2011). A large proportion of locally regulated PSR genes include those related to stress and hormone responses, including ethylene biosynthesis and signaling (Thibaud et al. 2010; see below). The regulation of these PSR genes appears to be tightly linked to root developmental changes (Thibaud et al. 2010), which include increases in root hair density and length (Bates and Lynch 1996, Ma et al. 2001), reduced activity of the primary root meristem (Ticconi et al. 2004, Sanchez-Calderon et al. 2005, Svistoonoff et al. 2007, Ticconi et al. 2009) and increased lateral root proliferation in regions of relatively high Pi availability (Linkohr et al. 2002, Lopez-Bucio et al. 2002, Reymond et al. 2006). A number of studies have investigated the role of ethylene in modifying root development in accordance with Pi availability. Pi deficiency has been

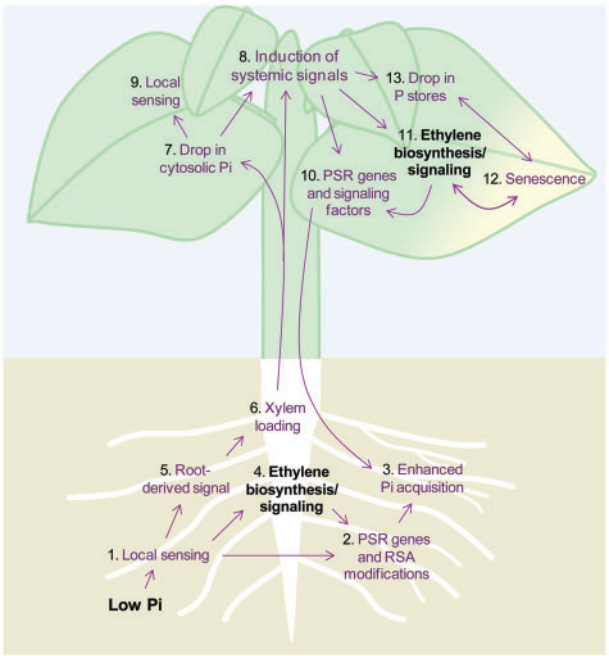

Fig. 1 Ethylene signaling is integrated into the Pi starvation response network. Low Pi is sensed locally, probably both extracellularly at the plasma membranes of root cells and intracellularly following uptake. Sensing of low Pi results in changes in Pi starvation response (PSR) gene expression and modifications in root system architecture (RSA), which aid in enhancing Pi acquisition. These responses are modulated, in part, by enhanced ethylene biosynthesis and responsiveness. The root-derived low Pi signal must be loaded into the xylem for transport to the shoot. This signal, or a decline in Pi itself, can lead to a drop in cytosolic Pi levels in the shoot, which elicits local, shoot signaling factors. In addition, the low Pi signals induce shoot-derived, systemic signaling factors such as PHR1 to enhance the expression of several downstream PSR genes. The expression of some PSR genes is regulated, in part, via changes in ethylene biosynthesis and responsiveness. The initiation of systemic signals also leads to a drop in P stores in leaves. Fluctuations in shoot P and ethylene signaling are interconnected with programmed senescence signaling pathways. In addition to regulating a number of responses in the shoot, systemically derived signaling factors, including microRNAs (e.g. miR399), move from shoot to root via the phloem to modulate PSRs in the root, many of which act to boost Pi acquisition.

shown to both promote ethylene synthesis (Borch et al. 1999, Lynch and Brown 2001, Li et al. 2009) and enhance ethylene responsiveness (He et al. 1992, Kim et al. 2008) in roots (**Fig. 1** [4]). Further support for these phenomena comes from analyses of transcript levels for ethylene biosynthetic genes, including those that encode 1-aminocyclopropane-1-carboxylate (ACC) synthases (*ACS*) and ACC oxidases (*ACO*), as well as members of the APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF) family of transcription factors, which are defined in part by their having a domain that binds ethylene-responsive elements (Nakano et al. 2006). Quantitative reverse transcription–PCR (qRT–PCR) analysis showed that transcripts for *ACS2*, *ACS4* and *ACS6* were elevated in Pi-deprived Arabidopsis roots (Lei et al. 2011). Transcriptome analyses of Pi-starved root tissues have also shown increased transcript levels for ethylene biosynthetic genes (Misson et al. 2005, Thibaud et al. 2010, Chacon-Lopez et al. 2011), as well as both up- and down-regulated *ERF* genes, in a variety of species (Wang et al. 2002, Misson et al. 2005, Hernandez et al. 2007, Zheng et al. 2009, Thibaud et al. 2010). Differences in apparent induction or repression among *ERF* genes could be due to the different species and experimental conditions utilized in the studies, and/or may reflect different roles for the various ERFs in Pi signaling. Split-root transcriptome analyses of Pi-starved Arabidopsis demonstrated that the transcript abundance of *ACO4* and several *ERF* genes increased in response to local Pi signaling mechanisms (Thibaud et al. 2010). We observed increased transcript abundance of *EIN3-BINDING F-BOX* (*EBF2*) in Pi-starved Arabidopsis roots (10-fold) and shoots (3-fold) via qRT–PCR analysis (V. K. Nagarajan and A. P. Smith, unpublished observations). *EBF2* promotes the degradation of ETHYLENE INSENSITIVE 3 (EIN3) and EIN3-LIKE 1 (EIL1), which are positive regulators of a variety of downstream ethylene responses (Potuschak et al. 2003, Binder et al. 2007). Together these results indicate that both ethylene biosynthesis and responsiveness are involved in local Pi sensing, although systemic signals are also integrated with ethylene signaling (see below). The data also highlight the complexity associated with the integration of ethylene synthesis and signaling in response to fluctuating Pi levels.

Recently, it was shown that the abiotic stress regulator, *FIERY1* (*FRY1*), is required for local regulation of some *Pht1* genes in the root (Hirsch et al. 2011). Under Pi-rich conditions, the *fry1* mutant exhibited increased transcript abundance for several *Pht1* genes in roots compared with the wild type, but not in shoots (Hirsch et al. 2011). Further experimentation suggested that loss of *FRY1* resulted in the derepression of *Pht1* expression via a local drop of Pi in the root stele, independent of systemic PSR signaling (Hirsch et al. 2011). FRY1 is a bifunctional enzyme that has inositol polyphosphate 1-phosphatase activity, as well as 3′,(2′),5′-bisphosphate nucleotide phosphatase activity, which catalyzes 3′-polyadenosine 5′-phosphate to AMP and Pi (Quintero et al. 1996, Hirsch et al. 2011). It appears that a defect in the nucleotide phosphatase activity is responsible for the local depletion of Pi levels (Hirsch et al. 2011). Interestingly, mutation of *FRY1* results in some ethylene-related phenotypes including decreased hypocotyl elongation in low light and various root system architecture alterations (see below; Chen and Xiong 2010). Microarray analysis of another mutant allele of *FRY1*, *rotunda1-1* (*ron1-1*), showed increased transcript abundance for at least 74 genes belonging to the Gene Ontology (GO) category 'response to ethylene stimuli', including *EBF2* (Robles et al. 2010). FRY1 has been implicated in several abiotic stress response pathways, such as cold, drought and salt (Xiong et al. 2001, Xiong et al. 2004). FRY1 may also function in low Pi stress responses by affecting local Pi sensing, possibly in conjunction with ethylene signaling.

Root hair development

In most plants, one of the earliest root morphological adaptations to Pi deficiency is a dramatic increase in root hair number and length within 1–2 d of Pi starvation (Bates and Lynch 1996, Ma et al. 2001, Jain et al. 2007). Studies employing ethylene signaling mutants and precursors/inhibitors of ethylene biosynthesis or signaling have demonstrated that ethylene modulates root hair growth in response to low Pi (Zhang et al. 2003, He et al. 2005). As with Pi starvation, treatment with the ethylene precursor, ACC, promotes root hair elongation, whereas inhibitors of ethylene synthesis and action decrease root hair elongation (Zhang et al. 2003). A number of ethylene response mutants had shorter root hairs than the wild type under low Pi conditions, but collectively had root hairs with lengths comparable with those of the wild type under high Pi conditions (Cho and Cosgrove 2002, Zhang et al. 2003). This indicates that Pi availability impacts ethylene responsiveness. Closer examination of ethylene's effect on Pi-dependent changes in root hair development indicates that ethylene enhances root hair density by shortening trichoblast cells to increase the number of H cells per unit length, and by increasing the number of H cells that form hairs, but not by increasing trichoblast cell file number—an adaptation that may involve auxin (Zhang et al. 2003). Although the development of extra root hairs in response to Pi deficiency does not appear to require ethylene signaling, a low Pi signal may directly activate primary ethylene response genes involved in epidermal cell differentiation (Schmidt and Schikora 2001).

Root development

Upon perception of low Pi, plants decrease primary root growth and increase lateral root development, forming a shallow root system to mine available Pi from soil (Linkohr et al. 2002, Lopez-Bucio et al. 2002, Ticconi et al. 2004, Sanchez-Calderon et al. 2006, Svistoonoff et al. 2007). These responses are largely regulated locally, independent of shoot Pi status (Svistoonoff et al. 2007, Ticconi et al. 2009). By altering ethylene biosynthesis or perception, several studies have demonstrated a role for ethylene in modulating Pi-starvation-responsive root growth (Borch et al. 1999, Lopez-Bucio et al. 2002, Ma et al. 2003,

Kim et al. 2008, Chacon-Lopez et al. 2011). Although the precise roles of ethylene in regulating root adaptations associated with Pi availability are difficult to dissect, it appears that ethylene regulates root elongation via changes in both biosynthesis and responsiveness. The specific response elicited depends not only on Pi availability, but also on root type (i.e. primary or lateral). Treatment of Arabidopsis seedlings with ACC under high Pi conditions resulted in meristem exhaustion of the primary root, mimicking a low Pi response, whereas inhibition of ethylene biosynthesis or signaling, with aminoethoxyvinylglycine (AVG) or Ag^+, respectively, restricted the low-Pi-induced meristem exhaustion (Chacon-Lopez et al. 2011). However, Ma et al. (2003) demonstrated that AVG treatment increased root elongation under high Pi conditions, but limited growth during Pi deficiency. Further, ethylene-insensitive mutants of tomato and petunia were deficient in low-Pi-induced adventitious root formation (Kim et al. 2008). Together, these studies indicate that ethylene plays a complex role in Pi-dependent root development in which it may restrict elongation of primary roots, but promote elongation of lateral roots. Although a number of Arabidopsis ethylene-insensitive mutants were shown to have more lateral roots than the wild type during Pi deficiency, ethylene's role in the initiation of lateral roots is not clear (Lopez-Bucio et al. 2002). It is possible that ethylene indirectly affects Pi-starvation-induced lateral root formation through interactions with auxin (Lopez-Bucio et al. 2002, Perez-Torres et al. 2008). Mutation of *FRY1* leads to reductions in lateral root initiation and elongation, as well as a decrease in primary root elongation through a defect in meristem maintenance and/or activity (Chen and Xiong 2010, Hirsch et al. 2011). The *fry1* mutant had reduced auxin responsiveness with regard to the lateral root initiation phenotype (Chen and Xiong 2010), but not the primary root phenotype (Hirsch et al. 2011). This further implicates a role for auxin in lateral root initiation, but a role for ethylene in primary root elongation. In addition to its root phenotypes, the *fry1* mutant exhibits lower sensitivity to ethylene inhibition of hypocotyl elongation, and introduction of the *ebf2* mutation into *fry1* restores this insensitivity (Chen and Xiong 2010). It is of interest to determine whether loss of *EBF2* in the *fry1* mutant background also restores its primary root phenotype.

In summary, ethylene plays an important role in Pi-dependent changes in root morphology. In response to low Pi conditions, both ethylene biosynthesis and responsiveness are enhanced (**Fig. 1**). Ethylene may play a relatively minor role during the early stages of low-Pi-induced changes in root development (i.e. initiation of new root hairs and lateral roots), but is necessary for the full elaboration of Pi-starvation-induced root modification by regulating the elongation of primary and lateral roots, as well as root hair growth. Recently we showed that constitutive overexpression of Arabidopsis *Pht1;5* resulted in a shorter primary root and increased proliferation of root hairs under Pi-sufficient conditions (Nagarajan et al. 2011). Interestingly, treatments with ethylene biosynthetic (AVG) and perception (Ag^+) inhibitors rescued these primary root and root hair phenotypes, respectively, suggesting modulation of ethylene signaling in these lines (Nagarajan et al. 2011). This, along with the observation that the *Pht1;5* overexpressors also accumulated less Pi in shoots and more in roots relative to the wild type (Nagarajan et al., 2011), may indicate that a disruption of the shoot : root Pi ratio triggers alterations in ethylene biosynthesis and/or signaling.

Systemic Pi responses

Systemic Pi signaling

The information of Pi depletion at the roots is passed to the shoots via unknown transmission machinery. Split-root experiments with roots divided between Pi-sufficient and -deficient medium showed that Pi must be translocated to the shoot to down-regulate the expression of PSR genes (C. Liu et al. 1998, Burleigh and Harrison 1999). The components and underlying mechanisms of this long-distance signaling have not been elucidated, but may involve Pi itself, strigolactones and cytokinins (**Fig. 1** [5]; Chiou and Lin 2011). Arabidopsis *PHOSPHATE 1* (*PHO1*) is involved in loading Pi from the root epidermal and cortical cells into the xylem (Poirier et al. 1991, Hamburger et al. 2002). Due to a defect in Pi transfer to the shoots in *pho1* mutant plants, PSR genes are strongly expressed in the shoots even under Pi-replete conditions (Poirier et al. 1991). Recent evidence suggests that PHO1 may also be involved in the translocation of a signaling molecule other than Pi from roots to shoots (Rouached et al. 2011). Thus, *PHO1* appears to play an important role in transduction of root-generated low Pi signals to shoots (**Fig. 1** [6]).

The transmission of low Pi signals to shoots and decreases in cytosolic Pi (**Fig. 1** [7]) can initiate shoot systemic (**Fig. 1** [8]) and local Pi sensing signals (**Fig. 1** [9]), respectively (**Fig. 1**; Bustos et al. 2010, Thibaud et al. 2010, Rouached et al. 2011). Evidence over the past decade points toward multifaceted regulation of PSR gene expression in plants (**Fig. 1** [10]; Vance 2010, Chiou and Lin 2011). The first systemic Pi regulator identified was the Arabidopsis myeloblastosis (MYB)-like transcription factor PHOSPHATE STARVATION RESPONSE 1 (PHR1), which has been shown to play an integral role in modulating responses to Pi starvation (Rubio et al. 2001, Bari et al. 2006, Nilsson et al. 2007, Bustos et al. 2010). Orthologs of PHR1 have been found in rice (Zhou et al. 2008) and common bean (Valdes-Lopez et al. 2008). PHR1 is predicted to bind a consensus PHR1-binding *cis*-element found in the promoters of numerous PSR genes (Rubio et al. 2001, Bustos et al. 2010). In addition to regulating downstream, structural PSR components, PHR1 modulates other transcription factors and signaling components. A number of recent studies have focused on the role of the micro-RNA miR399 in low-Pi-induced systemic signaling downstream of PHR1. Six species of miR399 and their primary transcripts are strongly up-regulated to varying extents by Pi deficiency in Arabidopsis

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(Fujii et al. 2005, Aung et al. 2006, Bari et al. 2006, Chiou et al. 2006). miR399 species promote Pi acquisition by directing cleavage of a ubiquitin E2 conjugase (*UBC24* or *PHO2*) mRNA, which encodes a negative regulator of Pi transport in the roots (Aung et al. 2006, Bari et al. 2006, Chiou et al. 2006). Further, analysis of phloem sap of cucurbits and Arabidopsis revealed that *miR399* species act as mobile signaling molecules facilitating communication between the shoot and root (Lin et al. 2008, Pant et al. 2008). In addition to miR399, a number of other components have been implicated in transmitting Pi signals to roots, including photosynthetic carbon assimilates, other small RNA species and Pi itself (**Fig. 1** [10]; Chiou and Lin 2011).

Ethylene's role in systemic Pi responses

Recent evidence has suggested that, as with local Pi mechanisms in roots, shoot systemic Pi signaling is modulated in part by ethylene (**Fig. 1** [11]). As in roots, ethylene synthesis is promoted in shoots in response to Pi deprivation (Kim et al. 2008). Transcriptome analysis of Pi-starved Arabidopsis leaves revealed increased transcript levels for the ethylene biosynthetic genes, *ACS6*, *ACS7* and *ACO4* (Misson et al. 2005). Similarly, Morcuende et al. (2007) detected increases in *ACS2* and *ACS6* transcripts in Pi-starved whole Arabidopsis seedlings. Notably, upon resupply of Pi, the observed increases in *ACS2* and *ACS6* transcripts were reversed to levels similar to those found in Pi-sufficient tissues (Morcuende et al. 2007), indicating a strong correlation between ethylene biosynthesis and Pi availability. Transcriptome analysis of a double knockout mutant of Arabidopsis *PHR1* and its partially redundant ortholog, *PHR1-LIKE 1* (*PHL1*), resulted in attenuation of the low Pi induction of *ACS6* and *ACS7* (Bustos et al. 2010). Further, by treating plants carrying a post-translationally controlled PHR1 overexpression construct with inducer and cycloheximide simultaneously, Bustos et al. (2010) identified several hundred genes that are likely to be direct targets of PHR1, and *ACS7* was among them. Interestingly, *ACS2* expression appears to be independent of PHR1 and/or PHL1 (Bustos et al. 2010), suggesting that the regulation of only a subset of ethylene biosynthetic genes is interconnected with systemic Pi signaling pathways. It is tempting to speculate that the Pi-dependent regulation of ethylene biosynthesis is modulated distinctly by local and systemic sensing pathways via regulation of different ethylene biosynthetic genes. In addition to controlling ethylene biosynthesis, PHR1 appears to regulate the transcription of a number of AP2/ERF genes (Bustos et al. 2010). Transcriptome analysis showed that the low Pi induction of at least eight AP2/ERF genes is attenuated in the *phr1 phl1* double mutant (Bustos et al. 2010). However, only one of these was predicted to be a direct target of PHR1, and the transcript abundance of many more AP2/ERF loci was not sensitive to knockout of *phr1* and *phl1* (Bustos et al. 2010). Together these data indicate that both ethylene biosynthesis and responsiveness are interconnected with local and systemic Pi signaling pathways.

High-affinity Pi transporters

Among the most prominent systemically controlled responses to Pi deprivation are factors aimed at enhancing Pi acquisition from soil and remobilizing P from existing metabolites, such as RNA, organic phosphates and phospholipids (Thibaud et al. 2010). The Arabidopsis Pht1;1 and Pht1;4 transporters play a key role in acquisition of Pi from soil. Expression analyses suggest that Pht1;4, along with Pht1;5, is also involved in Pi translocation throughout the plant (Mudge et al. 2002, Misson et al. 2004, Shin et al. 2004, Nagarajan et al. 2011). Recently, Lei et al. (2011) used a forward genetics screen to isolate a low-Pi-sensitive mutant of Arabidopsis, *hps2* (*hypersensitive to Pi starvation 2*), that they found to be a new mutant allele of *CTR1* (*CONSTITUTIVE TRIPLE RESPONSE 1*), a rapidly accelerated fibrosarcoma (RAF)-like kinase that is a key negative regulator of ethylene signaling (Kieber et al. 1993). qRT–PCR experiments revealed that transcript levels for *Pht1;1* and *Pht1;4*, were elevated in *hps2* under both low and high Pi conditions compared with the wild type (Lei et al. 2011). Also, expression of *Pht1;1* and *Pht1;4* was decreased in the ethylene-insensitive mutant, *ein2-5*, under low Pi conditions (Lei et al. 2011). Treatment with the ethylene precursor ACC induced *Pht1;4* expression, whereas Ag^{+} repressed *Pht1;4* expression. Notably, a lower concentration of ACC was able to elicit a response under low Pi conditions than under Pi sufficiency, indicating enhanced ethylene responsiveness during Pi deficiency (Lei et al. 2011). In a recent study using *Medicago falcata*, ACC induced expression of two *Pht1* genes, *MfPT1* and *MfPT5*, under Pi-sufficient conditions, whereas AVG and Co^{2+}, two ethylene synthesis inhibitors, each blocked the low-Pi-induced expression of these genes (Li et al. 2011). Together these studies indicate that ethylene is involved in regulating the expression of high-affinity Pi transporters.

Pi recycling

Acid phosphatases recycle Pi from a broad range of organic P compounds, and can act both on intracellular compounds or extracellularly, following secretion into the rhizosphere (Lefebvre et al. 1990, Li et al. 2002, Wang et al. 2011). Similar to expression of *Pht1* loci, expression of the *MfPAP1* acid phosphatase from *M. falcata* is up-regulated by ACC and down-regulated by AVG (Li et al. 2011). Likewise, transcript levels for Arabidopsis *ACID PHOSPHATASE TYPE 5* (*ACP5*; del Pozo et al. 1999) were elevated in the *hps2* mutant but reduced in *ein2-5* (Lei et al. 2011). Importantly, in both of the studies cited above, the changes in acid phosphatase transcript levels correlated with changes in acid phosphatase activity. These activities appeared to be associated with both endogenous acid phosphatases and those that are secreted into the external medium (Lei et al. 2011, Li et al. 2011). Similarly to acid phosphatases, RNases are involved in the remobilization of metabolic P, but via degradation of RNAs (Bariola et al. 1994). Transcript levels for the low-Pi-inducible *RIBONUCLEASE 1* (*RNS1*) gene were higher in *hps2*, but lower

in *ein2-5* under both low and high Pi conditions (Lei et al. 2011). Surprisingly, transcript levels for *RNS1* and *ACP5* were lower in a transcriptome analysis of the *ctr1-2* mutant as compared with the wild type (Brodersen et al. 2006). These apparent discrepancies between *hps2* and *ctr1-2* (i.e. two alleles of *ctr1*) could result from different tissues being analyzed. The qRT–PCR analyses of *hps2* utilized young seedlings, whereas the *ctr1-2* microarray used RNA isolated from rosette leaves of mature plants. These results may also reveal a complex involvement of ethylene in the regulation of Pi-scavenging genes that integrates both environmental and developmental cues. For example, *RNS1*, *ACP5* and ethylene all play a role in programmed senescence (see below).

Pi distribution

Recent studies have revealed that complex post-transcriptional mechanisms are involved in regulating internal Pi distribution. One such mechanism involves Pi-starvation-responsive miR399-directed cleavage of *PHO2* (*UBC24*), which results in de-repression of *Pht1* genes and a concomitant increase in Pi acquisition and translocation to the shoots (Aung et al. 2006, Bari et al. 2006, Chiou et al. 2006). Pi-starvation-induced ribo-regulators, *At4* and *IPS1*, act as 'target mimics' by imperfectly base-pairing with miR399 and tempering its effect on *PHO2* mRNA (Franco-Zorrilla et al. 2007). This tightly regulated circuit ensures efficient allocation of Pi to cells and prevents excessive Pi accumulation that could result in Pi toxicity. qRT–PCR experiments revealed that during Pi deficiency, *At4* and *miR399d* transcript levels were higher in the *hps2* mutant compared with the wild type, but were lower in *ein2-5*, suggesting that ethylene signaling interferes with shoot to root Pi transmission (Lei et al. 2011). Interestingly, transcript levels for *IPS1* were decreased in *ein2-5*, but were unchanged in *hps2* (Lei et al. 2011). This may reflect that the two key Arabidopsis ribo-regulators involved in *miR399* suppression, namely *At4* and *IPS1*, are regulated by distinct ethylene-dependent pathways and/or have different degrees of responsiveness to ethylene.

When starved for Pi, plants disproportionately allocate resources, including sugars and Pi, to roots to allow for continued mining of soil Pi (Hammond and White 2011). This results in an increase in root : shoot biomass. Exposure of plants to salinity has a similar impact, in which shoot growth is inhibited while root growth is maintained (Munns and Tester 2008). Studies have suggested a link between this disproportionate growth and ethylene biosynthesis. Tomato plants exposed to salinity for 2 weeks exhibited increased synthesis of ACC in both roots and shoots, but the levels in shoots were approximately 4-fold greater than in roots (Albacete et al. 2008). Also, transgenic canola expressing a bacterial ACC deaminase, which degrades ACC, did not experience the typical increase in root : shoot biomass ratio after salinity exposure (Sergeeva et al. 2006). Thus, the salinity-dependent increase in root : shoot biomass may result from greater ethylene production in shoots than in roots, which correspondingly leads to disproportionate shoot growth inhibition. Interestingly, tomato, as well as petunia, plants starved for Pi also produce more ethylene in shoots than in roots (Kim et al. 2008). Together these studies suggest that regulation of ethylene biosynthesis, which is influenced by Pi distribution, plays a role in increasing root : shoot biomass during Pi deficiency.

Senescence and other points of cross-talk

Programmed senescence results in recycling of Pi from older, dying organs to younger, meristematically active parts of the plant (**Fig. 1** [12, 13]; Buchanan-Wollaston et al. 2003, Lim et al. 2007). Ethylene has been shown to play an important role in regulating senescence of leaves, petals and fruits (Woltering and Vandoorn 1988, Wang and Woodson 1989, Abeles and Takeda 1990, Grbic and Bleecker 1995). In senescing petunia corollas, P levels drop by 75% (Verlinden 2003). In transgenic petunia overexpressing a mutated Arabidopsis ethylene receptor (*etr1-1*; Wilkinson et al. 1997), in which the plants have reduced sensitivity to ethylene, corolla senescence is delayed and P remobilization is decreased (Chapin and Jones 2009). Transcript abundance for the *Pht1* gene, *PhPT1*, was shown to increase in senescing corollas as P content decreased. Also, exogenous ethylene increased *PhPT1* expression, and this increase was blocked in the *etr1-1* petunia line (Chapin and Jones 2009). The ethylene inducibility of *PhPT1* was not affected by cycloheximide treatment, suggesting that *PhPT1* is a primary ethylene response gene (Chapin and Jones 2009).

In addition to their role in Pi starvation adaptation, Arabidopsis *RNS1* and *ACP5* appear to degrade metabolites during senescence to release Pi (Van der Graaff et al. 2006), which is then mobilized out of the cell by Pi transporters. Recently, we showed that *Pht1;5* overexpression lines senesced earlier than the wild type (Nagarajan et al. 2011). These plants also showed increased Pi transfer from older rosette leaves to siliques and increased transcript abundance of *RNS1* and *ACP5* (Nagarajan et al. 2011). Therefore, increased Pht1;5 activity may concomitantly enhance Pi scavenging to release more Pi, or, alternatively, the reduction of Pi due to enhanced Pht1;5 activity induces a subset of PSR genes. The rice OsPht1;8 transporter may play a similar role to Pht1;5 in senescence (Jia et al. 2011). Together these data suggest cross-talk among ethylene signaling, programmed senescence and P homeostasis.

In addition to senescence, many factors are likely to impact interactions between ethylene and Pi signaling, including other hormones, particularly auxin (Lopez-Bucio et al. 2002), cytokinin (Brenner et al. 2005), jasmonic acid (Chacon-Lopez et al. 2011) and strigolactones (Koltai and Kapulnik 2011); sugars (Hammond and White 2011); iron (Lingam et al. 2011, Wu et al. 2011); nitrate (Leblanc et al. 2008); inositol hexakisphosphate (Stevenson-Paulik et al. 2005); reactive oxygen species (Shin and Schachtman 2004, Tyburski et al. 2010); and mycorrhizal associations (Hayat et al. 2010, Lopez-Raez

et al. 2010, Mukherjee and Ane 2011). Future studies aimed at dissecting the relative importance of these components in Pi signaling pathways will aid in developing a better picture of how distinct Pi starvation responses are initiated and elaborated.

Concluding remarks

Ethylene plays an important role in modulating both local and systemic responses to low Pi. A decline in extracellular Pi results in enhanced ethylene biosynthesis and responsiveness in roots, which helps to remodel the root system architecture for increased Pi mining capability. Perception of low Pi in shoots also leads to enhanced ethylene biosynthesis and responsiveness, which impacts expression of many PSR genes that are regulated systemically. However, the interactions between Pi and ethylene signaling pathways are complex, and ethylene does not solely determine the extent of responses initiated by low Pi conditions. For example, Pi-starvation-induced gene expression is attenuated in the *hps2* mutant, but is not abolished completely. Also, elongation of roots and root hairs during Pi deficiency is influenced by ethylene signaling, but not the initiation of new lateral roots and root hairs. Hence, ethylene signaling may be utilized during the manifestation of Pi starvation responses as a way to fine-tune the adaptations. Future work should characterize low Pi induced ethylene fluxes, spatially and temporally, as well as investigate the molecular interactions among ethylene and Pi signaling components, such as between EIN3/EIL and Pi-related transcription factors.

Funding

A. Smith's laboratory is supported by Louisiana State University and the National Science Foundation (NSF IOS-1127051).

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